Effects of Irradiation on Mediterranean Fruit Flies (Diptera: Tephritidae): Emergence, Survivorship, Lure Attraction, and Mating Competition

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ABSTRACT Irradiation of puparia in Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), sterile insect release programs can negatively affect adult fly performance. Emergence, survivorship, lure attraction, and mating competition tests were performed on irradiated and unirradiated Mediterranean fruit flies in Hawaii. Unirradiated flies of the Vienna-7 (*tsl*) strain had higher emergence, flight ability, and survivorship compared with irradiated flies. In general, unirradiated flies were more responsive to trimedlure, but this effect was not consistent for all strains at every age. Laboratory strains, of both unirradiated and irradiated flies, responded to trimedlure at a younger age than wild flies, which may be a result of inadvertent selection for decreased development time in laboratory-reared flies. Mating competition tests with irradiated and unirradiated flies showed no significant differences. Costs associated with the irradiation process and the development of alternative control techniques are discussed.

KEY WORDS *Ceratitis capitata*, sterile insect technique, quality control, dominant lethal mutation, *tsl*

THE MEDITERRANEAN FRUIT FLY, *Ceratitis captitata* (Wiedemann), is a multivoltine, polyphagous (350 plant species) insect pest that could have a devastating economic impact if it were to become established in California or Florida (Robinson et al. 1986, Liquido et al. 1991, Metcalf 1994). In these states, the sterile insect technique (SIT) is used in a preventative release program to inhibit Mediterranean fruit fly colonization. The goal of SIT is for released sterile males to mate with any introduced wild females, resulting in the production of infertile eggs (Knipling 1955).

Eradication and control programs for the Mediterranean fruit fly must continuously improve the quality of released flies. Observations and tests of mating competitiveness have shown that SIT males are at a disadvantage with wild males when competing for wild females (Robinson et al. 1986, Shelly et al. 1994, Cayol et al. 1999, Lance et al. 2000), and flies used in SIT programs are currently released biweekly because of high mortality (Barry et al. 2002). The effects of different doses of irradiation on flies has been thoroughly studied in the past (Hooper 1970, 1989), with work focusing on sterility (Rendon et al. 1996), longevity and emergence (Zumreoglu et al. 1979), olfactory response (Galun et al. 1985), mating competitiveness (Holbrook and Fujimoto 1970, Katiyar and Ramirez 1970, Hooper and Katiyar 1971), intratree activity (Vargas et al. 1998), and dispersal (Wong et al. 1982).

This study establishes some of the biological costs of irradiation to determine the potential benefit of lowering the dosage or eliminating the irradiation process. Unirradiated and irradiated mass-reared flies were compared at different ages for attraction to trimedlure, and effects of irradiation on Vienna-7, *tsl* (temperature sensitive lethal) flies were evaluated for quality control parameters, including emergence, flight ability, adult survivorship, and mating competitiveness.

Materials and Methods

Quality Control Tests. Male Mediterranean fruit flies used in quality control tests were from the *tsl* all-male Vienna-7 strain and were obtained as puparia from the California Department of Food and Agriculture laboratory (Waimanalo, HI). Each lot of Vienna-7 flies (received 6 and 15 February and 8 March 2002) was partitioned so that one-half was irradiated (2 d before eclosion at 145 Gy at the USDA laboratory in Waimanalo) and one-half was not (i.e., unirradiated) (Rendon et al. 1996). Laboratory cages (model 1450BS, 30.5 by 30.5 by 30.5 cm; BioQuip, Gardena, CA) were used to measure emergence, flight ability, and survivorship for each treatment. Ten replicate cages for each lot were used to evaluate flight ability

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and emergence of irradiated and unirradiated flies (n = 100 puparia per cage). Survivorship of "flyers" was evaluated for three different diets (water + sucrose, water + sucrose with water removed on day 3, and water + sucrose with water and sucrose removed on day 3). (These diet regimens were used to simulate the diet of a typical SIT fly, which are provided with water and sucrose for 2–3 d before being released. In this experiment, the three treatments correspond to a released SIT fly finding water and sucrose, water only, and nothing, respectively.) All cages initially contained two sugar cubes, 50 ml of water, and a flight tube (black, hollow cylinder, 8.9 cm in diameter and 10 cm in height, coated with talcum powder to prevent flies from walking out of the tube) (Food and Agriculture Organization/International Atomic Energy Agency/United States Department of Agriculture 1998).

To evaluate the condition of nonfliers (flies unable to escape from the flight tubes), cages were examined 5 d after puparia irradiation (or 3 d after most flies eclosed). This interval is comparable to the timing with sterile fly releases in the California Preventative Release Program. The flight tubes were placed in a freezer to kill the flies, and after 2 h, the flies were examined and separated into four categories: uneclosed (UE), partially eclosed (PE; fly not fully separated from the puparium), deformed adult (DA), and flies appearing "normal" that were nonfliers (NF). Percentage of emergence was determined as (100 − [UE + PE]), and percentage of fliers was determined as (100 − [UE + PE + DA + NF]).

Fly mortality was recorded on days 3, 5, 7, 9, and 11 (day 0 was the day that most flies eclosed and was 2 d after irradiation) only for flies that escaped from the flight tube. Thus, survivorship tests corresponded only to this part of the cohort. All fly treatments had access to water and sugar until day 3, and then depending on treatment, flies were provided with water (6 February), water and sugar (15 February), or neither water nor sugar (8 March).

Data for emergence and flight ability (with lots pooled) and survivorship were analyzed using a two-way analysis of variance (ANOVA). Data were not transformed because responses fit normal distributions. Means were separated using Fisher least significant difference (LSD) test.

Lure Attraction. Males of four strains used in attraction tests were from the Maui-93 standard strain reared in the USDA laboratory in Waimanalo; the Vienna-4 *tsl* sexing strain reared at the USDA-ARS laboratory in Manoa, HI; the Hawaii-laboratory standard strain reared in the California Department of Food and Agriculture laboratory in Waimanalo; and wild flies collected as eggs and larvae from coffee (*Coffea arabica* L.) on Kauai, HI. For each laboratory strain, irradiated (2 d before eclosion at 145 Gy, at the USDA laboratory in Waimanalo) and unirradiated flies were obtained. Flies were evaluated at each age (1–14 d old) by using different cohorts of flies.

Circular, nylon-screened, field cages (2.5 m in height, 2.5 m in diameter) containing a 2- to 2.5-m tall rooted guava tree, *Psidium guajava* L., were used to test male attraction to a lure (Magnet Trimedlure 70–0 plug, 2 g of active material; Agrisense, Decatur, IL). Lures were placed in a dry bucket trap (1 liter in volume, with four entry holes of 2 cm diameter around the perimeter) containing a Vapona pesticide strip (Shell Chemical LP, Houston, TX). Cage tests involved placing 100 flies of the four strains, each marked with a different dye (Day-Glo Color, Cleveland, OH), into three or four field cages in Waimanalo, HI. Traps were checked 24 h later, and flies were scored using a UV light. Treatments (strain, age, and irradiation) were randomly assigned to cages. For each age, 14 replicates of the laboratory strains were evaluated; only 6 replicates of wild flies of each age were evaluated because they were less available. Tests were performed in a guava orchard at the University of Hawaii Experiment Station in Waimanalo, HI, between January 1997 and March 1999.

Data were analyzed using ANOVA, with strain, age, and irradiation means separated using Fisher LSD test (SAS Institute 1999). Within each strain, ANOVAs were used to compare the effects of irradiation at each age, and means were separated using Fisher LSD test. Data were not transformed because responses fit normal distributions.

Mating Tests. Male Mediterranean fruit flies used in mating tests were from the Vienna-7 *tsl* strain and were obtained as puparia from the California Department of Food and Agriculture laboratory in Waimanalo during February 2002. Flies were irradiated 2 d before eclosion at 145 Gy, at the USDA-APHIS mass-rearing facility in Waimanalo, and were marked with Day-Glo dye, which does not affect fly survival (Serghiou 1977). Female flies used in mating tests were obtained from T. Shelly's (USDA-APHIS) laboratory in Manoa, HI and were from a stock in the sixth generation of wild flies, collected as larvae from Jerusalem cherry (*Solanum pseudocapsicum* L.). To obtain virgin females for mating tests, newly emerged females were isolated from males <24 h after eclosion. These females were provided with water and fed honey and sugar with protein hydrolysate (in a 3:1 ratio) until they were sexually mature.

The cages used for mating trials were as described under Lure Attraction. In each cage, there were 50 irradiated males, 50 unirradiated males, and 50 females. A total of 10 replicates was evaluated in Waimanalo in February and March 2002. Both types of males were released into the cages first, followed by the females after an interval of 5–10 min. Flies that were dead, incapable of flight, or noticeably damaged in any way at the time of release were replaced. Two field observers located and removed mating pairs without fly replacement and scored marked males as irradiated or unirradiated. Observations were made from ≈0900 until 1300 h. A HOBO data logger (Pro Temp/RH; Onset Computer, Bourne, MA) was placed into one cage to record temperature and relative humidity. For a given replicate to be included in the analysis, at least 20% of the females had to be collected in mating pairs.

Table 1. Quality control data for unirradiated and irradiated Vienna-7 Mediterranean fruit fly strains (with three fly lots pooled, each treatment had 30 replicates)

QC parameter	Fly condition[a]		Effect of irradiation,[b] %	Statistics		
	Unirradiated	Irradiated		*F*	df	*P*
% Emergence	93.77 ± 0.46a	88.80 ± 0.79b	5	29.80	1,58	<0.0001
% Fliers	84.00 ± 1.59a	65.27 ± 1.64b	22	67.19	1,58	<0.0001
% Uneclosed (UE)	3.70 ± 0.38a	7.30 ± 0.63b	−97	23.96	1,58	<0.0001
% Partially eclosed (PE)	2.53 ± 0.27a	3.90 ± 0.37b	−54	28.02	1,58	0.004
% Deformed adults (DA)	6.80 ± 1.12a	17.60 ± 1.29b	−158	40.08	1,58	<0.0001
% "Normal" non-fliers (NF)	2.97 ± 0.40a	5.93 ± 0.75b	−100	12.18	1,58	0.001
% UE adjusted[c]	29.08 ± 3.30a	21.87 ± 2.01a	25	3.50	1,58	0.07
% PE adjusted[c]	18.13 ± 1.81a	11.72 ± 1.15b	35	8.93	1,58	0.004
% DA adjusted[c]	33.50 ± 3.87a	49.69 ± 1.83b	−48	14.29	1,58	0.0004
% NF adjusted[c]	19.29 ± 2.22a	16.72 ± 1.98a	13	0.74	1,58	0.4

[a] Values within rows with different lowercase letters are significantly different (Fisher's LSD test).
[b] Calculated using (1 − [irradiated/unirradiated]) × 100%.
[c] Data were adjusted for each replicate, by setting quality control parameters (UE + PE + DA + NF) equal to 1. Using ANOVAs, these frequencies were then analyzed and means were separated using Fisher's LSD test.

An ANOVA was used to compare data for the number of unirradiated and irradiated males that had mated, and means were separated using Fisher LSD test (SAS Institute 1999). Data were not transformed because responses fit normal distributions.

Results and Discussion

There were noticeable differences between unirradiated and irradiated laboratory flies with regard to quality control tests (emergence, flight ability, and survivorship) and lure attraction, but not in mating competition tests.

Quality Control Tests. The accepted quality control manual lists acceptable means for percentage of emergence and flyers for strains carrying a *tsl* mutation as 75 and 65% for unirradiated and 70 and 60% for irradiated flies, respectively (Food and Agriculture Organization/International Atomic Energy Agency/United States Department of Agriculture 1998). The effect of irradiation is assumed to lead to an ≈7% reduction in both emergence and flight ability ([75 − 70]/75 × 100% = 6.7%, and [65 − 60]/65 × 100 = 7.7%, respectively). In our study, irradiation resulted in a much higher reduction (22%) in flight ability (Table 1). The average number of unirradiated and irradiated flies not escaping from the flight tube was ≈16 and 35%, respectively. After adjusting quality control parameters (UE + PE +NF + DA = 1) for unirradiated and irradiated treatments, comparisons were made to see how they differed. The irradiation process resulted in lower adjusted values for UE, PE, and NF, and higher values for DA (Table 1).

Fly survivorship was different for the three diet treatments. At day 0, there were always more unirradiated fliers than irradiated fliers; however, these differences in survivorship only persisted when flies were provided with sugar and water (Fig. 1). When flies were supplied with water (only) after day 3 (Fig. 2) or nothing (Fig. 3), they all died quickly, with

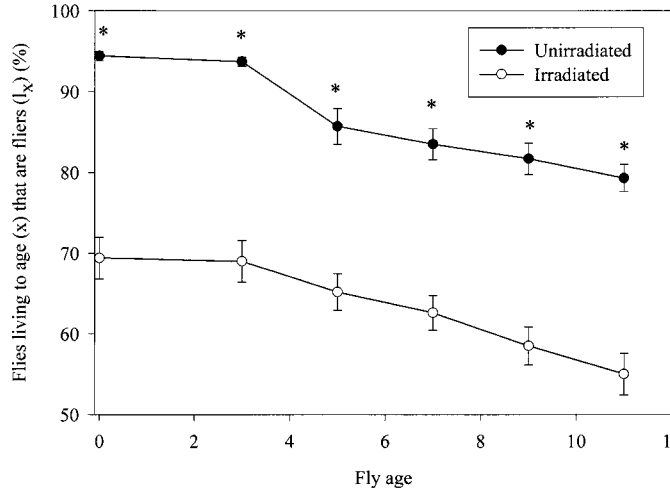

Fig. 1. Survivorship (mean ± SE) of unirradiated and irradiated Vienna-7 flies provided with water and sugar in laboratory cages. Only flies capable of escaping from the flight tube had access to food and water (*$P < 0.05$).

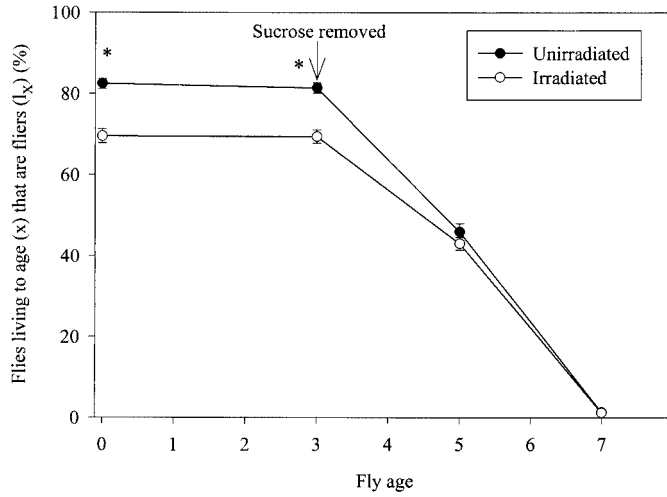

Fig. 2. Survivorship (mean ± SE) of unirradiated and irradiated Vienna-7 flies provided with water and sugar for 3 d after emergence and then only with water. Only flies capable of escaping from the flight tube had access to food and water (*$P <$ 0.05).

<5% living 1 wk. Data suggest there were no differences in survivorship for flies provided with water after day 3 in comparison with nothing provided. Thus, the cost of irradiation on fly survivorship seems to depend on adult diet. In the California Preventative Release Program, aerial releases are made twice a week (Dowell et al. 2000), probably because of low sterile fly survivorship. It cannot be assumed that this high mortality, which may seem comparable to flies in this study not feeding after day 3, is the result of sterile flies not finding good food sources, because other factors (i.e., predation) may be operating. The cost of irradiation may or may not persist under field conditions.

Lure Attraction. There were effects of strain (F = 6.77; df = 2,1159; P = 0.0012), irradiation (F = 32.49; df = 1,1159; $P < 0.0001$), and age (F = 24.92; df = 13,1159; $P < 0.0001$) on responses of flies to the trimedlure bait. For fly responses within a strain at each age, there was not always a significant effect of irradiation. Unirradiated flies demonstrated a greater response than irradiated flies for Vienna-4 at all ages, although the differences were only significant after day 7 (Fig. 4). The Hawaii-laboratory strain showed higher responses with unirradiated flies for the first 2 d and then similar responses across treatments afterward (Fig. 5). Unirradiated Maui-93 flies showed higher responses than did irradiated flies for ages

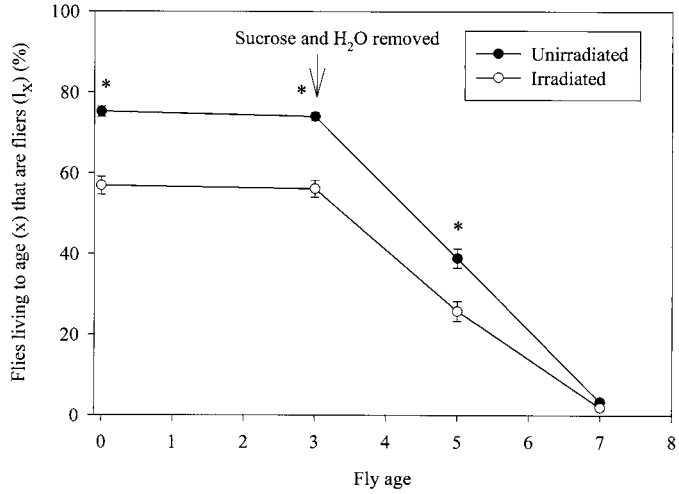

Fig. 3. Survivorship (mean ± SE) of unirradiated and irradiated Vienna-7 flies provided with water and sugar for 3 d after emergence and then with nothing. Only flies capable of escaping from the flight tube had access to food and water (*$P <$ 0.05).

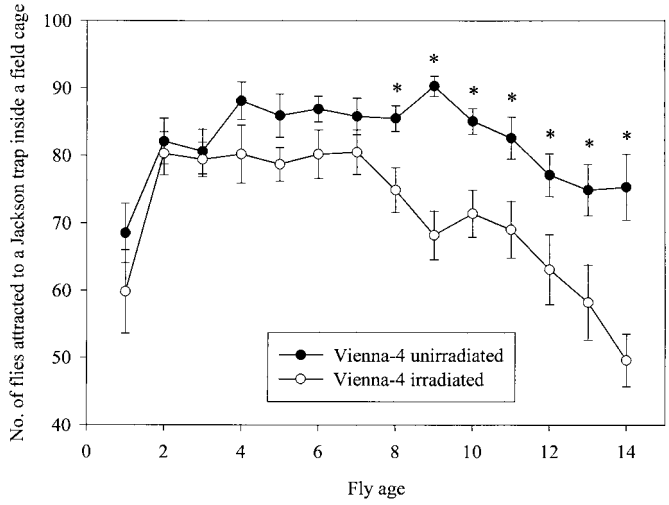

Fig. 4. Responses (mean ± SE) of unirradiated and irradiated Vienna-4 flies to trimedlure during a 24-h period at 14 different ages (*$P < 0.05$).

1–3 d, and subsequently, irradiated flies exhibited higher responses (Fig. 6). The effect of irradiation was significant at seven ages in Vienna-4 (Fig. 4); two ages in Hawaii-laboratory (Fig. 5); and at five ages in Maui-93 (Fig. 6). In general, there was a rapid increase in laboratory fly response for the first 4 d followed by a gradual decline, whereas wild flies had a gradual increase in lure response throughout the 14-d trial (Fig. 7). This difference may be the result of selection for decreased development time in laboratory-reared colonies (Cayol 2000).

Mating Tests. In mating competition tests, unirradiated males accounted for 54.4% of the matings, with an average of 15.0 ± 1.6 males mating, which was not significantly different from the 12.4 ± 1.3 irradiated males that mated ($F = 1.64$; df = 1,18; $P = 0.22$).

Based on the current study, the use of nonsterile laboratory flies would confer the greatest benefit to fly survivorship compared with their sterilized counterparts, providing flies could find adequate food sources. Even though the average irradiated male was found to be aspermic after mating with four females (Hooper and Katiyar 1971), whereas the average unirradiated male mated 14 times over its lifespan (Katiyar 1973), it is unlikely that SIT males used in the California Preventative Release Program will be required to mate more than once.

In an effort to increase the quality of flies used in SIT programs, several researchers have proposed reducing or even eliminating the irradiation process. Lowering the current irradiation dose (145 Gy) may be possible because most Mediterranean fruit fly SIT programs

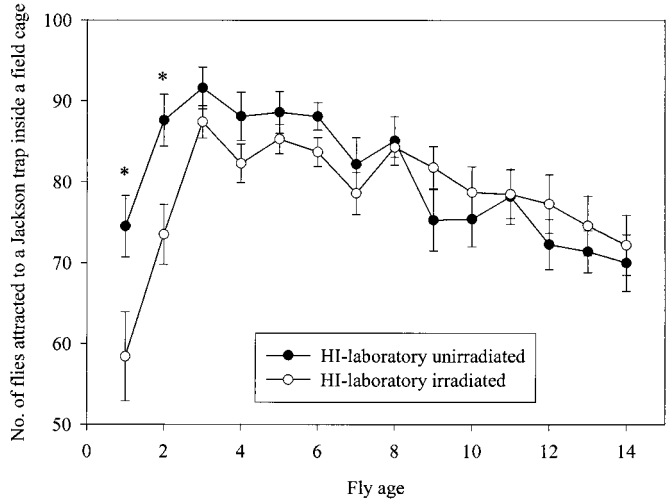

Fig. 5. Responses (mean ± SE) of unirradiated and irradiated Hawaii-laboratory flies to trimedlure during a 24-h period at 14 different ages (*$P < 0.05$).

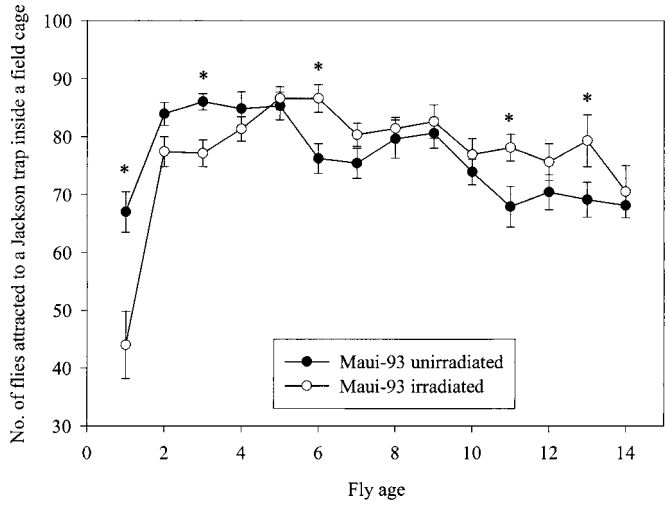

Fig. 6. Responses (mean ± SE) of unirradiated and irradiated Maui-93 flies to trimedlure during a 24-h period at 14 different ages (*$P < 0.05$).

now use laboratory strains that carry a *tsl* (temperature-sensitive lethal) mutation, which before irradiation, are semisterile from a Y-autosome chromosome translocation that produces all males (≈99%) (Steffens 1986). Strains carrying a *tsl* mutation also have a higher fitness cost, as shown by reduced emergence and flight ability in comparison to other laboratory strains (Rendon et al. 1996, Barry et al. 2002). Lowering irradiation dosages would not substantially change protocols for rearing centers and should lessen the observed differences in quality control parameters between unirradiated and irradiated flies. Before dosages could be lowered, research would need to be done evaluating different dosages and their effects on quality control parameters for the strains that are currently in use. This research has been done for the Vienna 42 strain carrying the *tsl* mutation in Guatemala (Rendon et al. 1996) and for the Hawaii-laboratory strain (E. B. Jang and D.O.M., unpublished data).

A genetic technique developed with *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) involves the release of insects carrying dominant lethal mutations (RIDL), in which all female progeny and one-half of all male progeny die (Heinrich and Scott 2000, Thomas et al. 2000). Incorporating RIDL into the present SIT programs would require more research and development, but it could potentially offer a much greater benefit. Some advantages of using RIDL include elimination of the sex-separation stage and the

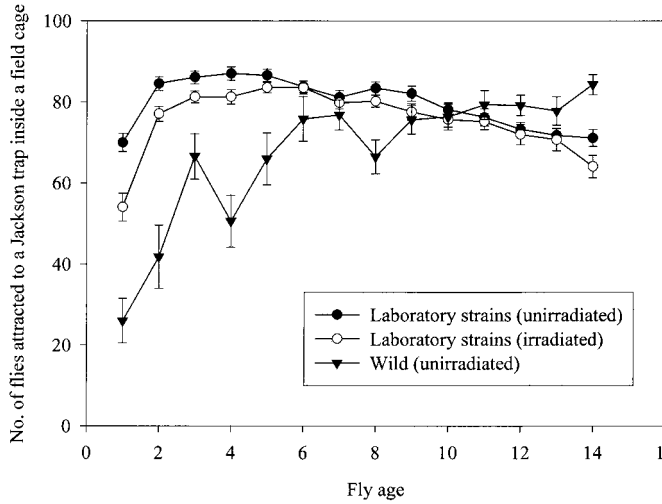

Fig. 7. Responses (mean ± SE) of unirradiated and irradiated laboratory (strains were pooled), and wild flies to trimedlure during a 24-h period at 14 different ages. At each age, $n = 42$ for unirradiated laboratory flies, $n = 42$ for irradiated laboratory flies, and $n = 6$ for wild flies (*$P < 0.05$).

entire irradiation process, the release of insects at any life-cycle stage, and fitness advantages of transgenic flies over their irradiated counterparts (Thomas et al. 2000). The cost of developing RIDL for Mediterranean fruit fly is not known, and the fitness cost (if any) of the alleles involved in RIDL system need to be determined for the Mediterranean fruit fly. However, the estimated annual costs associated with permanent establishment of the Mediterranean fruit fly in California are between $1.3 and $1.9 billion (Siebert and Pradhan 1991, Siebert and Cooper 1995, Siebert 1999, California Department of Food and Agriculture 2001). Such a promising enhancement to SIT, as the RIDL could be, should be developed for the Mediterranean fruit fly and evaluated in the field in the presence of wild populations to determine its future use.

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